Veterinary Parasitology, 26 (1988) 321-327
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Anthelmintic Activity of Continuous Low Doses of Fenbendazole into the Rumen of Sheep

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(Accepted for publication 23 February 1987)

ABSTRACT

Boisvenue, R.J., Colestock, E.L. and Hendrix, J.C., 1988. Anthelmintic activity of continuous low doses of fenbendazole into the rumen of sheep. *Vet. Parasitol.*, 26: 321-327.

Fenbendazole (FBZ) was continuously infused for 30 days into the rumen of 103 lambs which had mature or developing benzimidazole-susceptible or thiabendazole-resistant *Haemonchus contortus* and susceptible *Trichostrongylus colubriformis* infections. Ovicidal, larvicidal and adulticidal activities were exhibited against benzimidazole-susceptible and benzimidazole-resistant *H. contortus* worms by FBZ at a dose level of ≥ 0.2 mg kg^{-1} body weight day^{-1}. Reasonably consistent high level efficacy against *H. contortus* was obtained with dose levels > 0.4 mg kg^{-1} day^{-1}. Excellent control of susceptible *T. colubriformis* worms was achieved with the lowest dose tested of 0.4 mg kg^{-1} day^{-1}. The intraruminal infusion critical study method is a tool to determine the feasibility of incorporating a candidate anthelmintic compound in a continuous sustained-release rumen device formulation. The anthelmintic profile of FBZ obtained by low-level intraruminal administration suggests that it would be a potential candidate.

INTRODUCTION

Fenbendazole (Panacur; Hoechst) at therapeutic doses of 5 and 10 mg kg^{-1} body weight reduces helminth egg production (Baeder et al., 1974; Chroust, 1974; Kelly et al., 1975; Kirsch and Duwel, 1975) and removes immature parasitic stages (Duwel et al., 1974; Kennedy and Todd, 1975; Kirsch, 1974; Kirsch and Duwel, 1975; Ross, 1974). Prichard et al. (1978) suggested that the efficiency and spectrum of activity of different benzimidazole anthelmintics may be improved by extending the period during which parasites are exposed to effective drug concentrations. There have been several studies on the effect of single and divided dose administration on the efficacy of fenbendazole against stages of benzimidazole-resistant and non-resistant sheep trichostrongylids (Hogarth-Scott et al., 1976; Kelly et al., 1977; Hall et al., 1978; Sangster et al., 1979). A marked uniform improvement in efficacy was observed by Johnson

0304-4017/88/$03.50

(1979) when intrarumen dosing was carried out and compared to the oral route of treatment. McBeath et al. (1979) suggested in-feed worming of ewes around the time of lambing to avoid handling of animals which may lead to abortion or metabolic disorders. In the present study, the anthelmintic activity of fenbendazole was evaluated in sheep experimentally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* and treated by rumen infusion of the drug for 30 days.

MATERIALS AND METHODS

One hundred and three lambs of mixed breed and sex, weighing between 30 and 60 kg were used in six experiments. In the first experiment, 40 worm-free lambs were each experimentally infected with 10 000 anthelmintic-susceptible *H. contortus* infective larvae of United States Department of Agriculture (USDA) isolate BPL1 and allowed to reach maturity. Dose levels of fenbendazole (FBZ) of 0.042–0.800 mg kg^{-1} day^{-1} were continuously infused into the rumen of these lambs for 30 days using a surgically installed 1-inch rumen plastic cannula. Twenty-eight cannulated lambs were involved in the second experiment in which a single infection of 10 000 susceptible *H. contortus* larvae infection was given to worm-free lambs 24 h following initiation of rumen infusion of 0.400 and 0.600 mg kg^{-1} day^{-1}. In the third experiment, 17 cannulated lambs which had mature *H. contortus* infections were given in addition an oral inoculation of 10 000 susceptible larvae 24 h after starting rumen infusion of daily doses of 0.400–0.800 mg FBZ kg^{-1}. The forty-five lambs of Groups 2 and 3 were also inoculated simultaneously with 20 000 infective larvae of a USDA benzimidazole-susceptible isolate of *T. colubriformis*. Experiments 4 and 5 dealt with the effects of continuous infusion levels of 0.100–0.600 mg kg^{-1} day^{-1} given 24 h before thiabendazole (TBZ)-resistant *H. contortus* infections of 3 000 to 5 000 larvae daily for 5 days in 18 cannulated worm-free lambs. This isolate was obtained from Merck Co. in 1978 and was challenged in experimentally infected sheep in our laboratories with 66 mg TBZ kg^{-1} without success. Twelve worm-free cannulated lambs were infected only with susceptible *T. colubriformis* and allocated to Group 6. Continuous daily doses of 0.400–0.800 mg FBZ kg^{-1} were given for 30 days to lambs of Groups 2, 3 and 6 having either mature or developing *T. colubriformis* infections.

All test lambs were infused in a similar manner. A stock solution of FBZ was prepared from commercial 10% suspension (Panacur, Hoechst) diluted with sterile distilled water for proper concentration. The infusion suspension containing the appropriate amount of FBZ based on animal weight was prepared every 24 h. The total suspension was continuously agitated and was dispensed by a Harvard peristaltic pump (model No. 1203) at the rate of 0.5 ml min^{-1}. A group of cannulated infected lambs, infused with sterile distilled water, served as controls in each experiment. Animals having established infections were

TABLE I

Efficacy of fenbendazole (FBZ) against established benzimidazole-susceptible *Haemonchus contortus* in lambs treated by continuous rumen infusion for 30 days.

Experiment group	No. animals	Treatment	Daily dose (mg kg^{-1})	Eggs per gram feces			Group average worm counts	
				Treatment		Percentage reduction	Total	Percentage reduction
				Pre-	Post-			
1A	2	FBZ	0.042	100	350	0	403.5	58
1B	2	FBZ	0.084	400	300	25.0	465.0	52
1C	2	FBZ	0.167	784	50	93.6	0	100
1D	2	FBZ	0.358	3733	0	100	0	100
1E	8	FBZ	0.400	3278	25	99.2	168.2	82
1F	3	FBZ	0.600	756	0	100	0	100
1G	10	FBZ	0.800	5899	150	97.4	1.3	99
1H	11	None	-	2942	2900	1.4	959.2	-

examined for worm eggs in feces prior to the test. The McMaster fecal examination technique was conducted at the start and at weekly intervals during the test period. Feces of lambs infected with larvae following infusion were examined microscopically daily beginning 2 weeks post-infection in order to determine onset of patency. At the termination of a test, lambs were killed by electrocution and a total worm recovery was made from each animal. Parasites recovered were identified, sexed, sized and female worms were examined for viable ova. Animal weights were taken at the beginning and end of an experiment. Feed consumption was recorded every week to observe anorexia or effects of haemonchosis.

RESULTS

Data collected in the first experiment, in which lambs with drug-susceptible mature *H. contortus* infections were treated with titrated doses of FBZ, are given in Table I. Greater than 90% reduction of nematode egg production in the feces occurred in lambs infused with a daily dose of 0.167 mg FBZ kg^{-1} or higher. Reasonably consistent high-level efficacy against established *H. contortus* infections can be claimed for dose rates greater than 0.4 mg kg^{-1} day^{-1}. This dose level was very effective in removing worms before patency in lambs of Experiment 2 and removing mature worms from lambs in Experiment 3 (Table II). In addition, fecal worm egg production was greatly reduced. Ova in the uterus of female *H. contortus* retained in lambs which were dosed at ≥ 0.084 mg kg^{-1} day^{-1} were microscopically examined and found to be atypical in appearance. No third-stage larvae could be cultured from the feces of treated lambs. It was observed although not tabulated, that adult benzimida-

TABLE II

Efficacy of fenbendazole (FBZ) against various stages of *Haemonchus contortus* in lambs treated by continuous rumen infusion for 30 days

Experiment group	No. animals	Treatment	Daily dose (mg kg^{-1})	Eggs Per Gram Feces			Group average worm counts	
				Treatment		Percentage reduction	Total	Percentage reduction
				Pre-	Post-			
2A	6	FBZ	0.400	0	0	100	15.0	98.1
2B	12	FBZ	0.600	0	0	100	0	100
2C	10	None	-	0	8000	0	801.0	-
3A	4	FBZ	0.400	4193	200	95.2	35.0	96.1
3B	3	FBZ	0.600	2267	0	100	0	100
3C	6	FBZ	0.800	125	0	100	6.0	99.3
3D	4	None	-	600	2600	0	907.0	-

TABLE III

Efficacy of fenbendazole (FBZ) against developing stages of thiabendazole (TBZ)-resistant *Haemonchus contortus* in lambs treated by continuous rumen infusion for 30 days

Experiment group	No. animals	Treatment	Daily dose (mg kg^{-1})	Eggs per gram feces			Group average worm counts	
				Treatment		Percentage reduction	Total	Percentage reduction
				Pre-	Post-			
4A	2	FBZ	0.500	0	0	100	49.5	97.9
4B	2	FBZ	0.600	0	0	100	1.0	99.9
4C	2	TBZ	0.500	0	2300	0	3825.5	0
4D	2	None	-	0	1150	0	2442.5	-
5A	2	FBZ	0.400	0	0	100	15.0	99.7
5B	2	FBZ	0.300	0	0	100	42.5	99.1
5C	2	FBZ	0.200	0	0	100	41.0	99.1
5D	2	FBZ	0.100	0	800	48.4	1511.0	66.4
5E	2	None	-	0	1550	0	4499.0	-

zole-susceptible worms found at necropsy of lambs following the low-level infusion were markedly stunted in length. Complete inhibition of worm egg production and > 98% reduction in worm population were obtained in lambs given thiabendazole-resistant *H. contortus* larvae simultaneous with ≥ 0.2 mg kg^{-1} day^{-1} FBZ infusion (Table III). Thiabendazole was ineffective against the Merck isolate in lambs treated daily with 0.5 mg kg^{-1} for 30 days.

TABLE IV

Efficacy of fenbendazole (FBZ) against various stages of benzimidazole-susceptible *Trichostrongylus colubriformis* in lambs treated by continuous rumen infusion for 30 days

Experiment group	No. animals	Treatment	Daily dose (mg kg^{-1})	Eggs per gram feces			Group average worm counts	
				Treatment		Percentage reduction	Total	Percentage reduction
				Pre-	Post-			
2A	6	FBZ	0.400	0	0	100	217	96.7
2B	12	FBZ	0.600	0	0	100	0	100
2C	6	None	-	0	2600	0	6712	-
3A	4	FBZ	0.400	138	0	100	0	100
3B	3	FBZ	0.600	111	0	100	0	100
3C	6	FBZ	0.800	125	0	100	6	95.1
3D	4	None	-	76	0	100	202	-
6A	4	FBZ	0.400	100	0	100	0	100
6B	4	FBZ	0.800	600	0	100	0	100
6C	4	None	-	400	100	25	2821	-

Efficacy data against developing and mature stages of benzimidazole-susceptible *T. colubriformis* infections in lambs are summarized in Table IV. Daily dose levels of 0.600 and 0.800 mg FBZ kg^{-1} completely removed worms prior to patency or eliminated mature *T. colubriformis* worms in lambs of experimental Groups 2, 3 and 6. After 7 days of infusion, it was observed that worm eggs had disappeared in the feces of lambs of these three groups. There were no signs of toxicity in any test lambs.

Anemia due to haemonchosis appeared in several experimentally infected lambs prior to infusion treatment. A dramatic improvement in the health of affected lambs, i.e. loss of anemia, was seen after 7 days of infusion. It was observed at this time that the number of worm eggs per gram of feces was markedly reduced in lambs treated with ≥ 0.4 mg FBZ kg^{-1} day^{-1}.

DISCUSSION

Kirsch and Schleich (1982) reported that FBZ exhibited ovicidal activity against trichostrongylid eggs in lambs treated with doses of 0.1 mg kg^{-1} for 4 days. In our studies, FBZ at levels of ≥ 0.167 mg kg^{-1} day^{-1} for 30 days greatly reduced or eliminated the number of *H. contortus* and *T. colubriformis* eggs in

the feces of lambs after 7 days infusion. Ova in utero of female worms recovered at necropsy of lambs dosed at 0.084 mg kg^{-1} day^{-1} were atypical. Prichard et al. (1978) reported that the effectiveness of a benzimidazole anthelmintic is correlated with the duration that the parasites are exposed to high concentrations. Approximately 99% of thiabendazole-resistant *H. contortus* worms were removed in lambs treated by intraruminal administration at low dose concentrations of ≥ 0.2 mg kg^{-1} day^{-1}. In a subsequent review by Prichard (1985), it was reported that about 30% FBZ passed through the rumen unabsorbed, whereas only about 12% TBZ did so. The slower absorption of FBZ from the rumen provides a reservoir which helps to prolong the period of high circulating levels. The effect is to subject drug-tolerant worms to longer periods of exposure to the FBZ treatment. Although the comparison behavior study of single doses of FBZ and TBZ was conducted in cattle, it may follow that FBZ behaves in a similar manner in sheep. Continuous exposure and accumulation of unabsorbed FBZ in the rumen by daily infusion may account for the marked effect of this benzimidazole compound against thiabendazole-resistant *H. contortus*. In addition, this marked effect at continuous low doses of FBZ aided in the control of haemonchosis in lambs.

Ludwig and Boisvenue (1980) described a controlled release rumen device (RDD) formulation for compounds that have anthelmintic properties of ovicidal, larvicidal and adulticidal activity at a continuous low dose. The intraruminal infusion of therapeutically active compounds at very low doses in parasitized lambs treated over 30 days provides data to determine the feasibility of candidate compounds in RDD. Properties of fenbendazole observed in these studies suggest that FBZ would be a candidate compound for incorporation in a controlled RDD formulation for cattle and sheep.

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